ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES

DEPARTMENT OF MEDICAL LABORATORY SCIENCES

Mother to Child Transmission of Hepatitis B Virus and Associated Factors at Government Hospitals, in Addis Ababa, Ethiopia.

By: Kidist Birhanu (BSc)

Advisors: Dr. Abay Sisay (MSc, PhD)

Regasa Diriba (BSc, MSc)

Collaborator: Gadissa Gutema (BSc, MSc, PhD Cand.)

A research thesis submitted to the Department of Medical Laboratory Sciences, College of Health Science, Addis Ababa University, in partial fulfillment of Master of Sciences in Clinical Laboratory Sciences, (Diagnostic and Public Health Microbiology specialty).

June 2024

Addis Ababa, Ethiopia

Addis Ababa University

Department of Medical Laboratory Sciences

This is to certify that the thesis prepared by Kidist Birhanu Deressa entitled: Mother to Child Transmission of Hepatitis B Virus and Associated Factor in Government Hospital in Addis Ababa, Ethiopia from April 2023 to May 2024 a Cohort Study and submitted in partial fulfillment of the requirements for Master of Sciences in Medical Laboratory Sciences, Diagnostic, and Public Health Microbiology specialty. complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

Signed by the Examining Committee:

External Examiner_____Signature _____Date_____

Internal Examiner_____Signature_____Date_____

Advisor_____Signature _____Date_____

Advisor_____Signature_____Date_____

Collaborator_____Signature_____Date_____

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Chairman of the Department of Graduate Program Coordinator

Acknowledgment

I want to express my deepest gratitude to the Almighty God for giving me the strength and courage to develop this research. I am truly grateful for His guidance and assistance throughout this endeavor.

I gratefully acknowledge Addis Ababa University, College of Health Science, Department of Medical Laboratory Sciences for providing this opportunity to perform my thesis work.

I sincerely appreciate my advisors, Dr. Abay Sisay and Mr. Regasa Diriba for their invaluable guidance and support. I am truly fortunate to have had their expertise and mentorship throughout this research. I am grateful for the time they dedicated to reading through my work and their tireless efforts in providing insightful comments and suggestions.

I would also like to thank Mr. Gadissa Gutema for allowing me to participate in this project study. His support and encouragement have shaped my understanding of the subject matter.

I extend my thanks to the staff and administration of Abebech Gobena Hospital, Alert Hospital, Federal Police Hospital, Dagmawi Minilik II Hospital, Gandi Memorial Hospital, Ras Desta Damtew Memorial Hospital, St, Paul Hospital Millennium Medical College, St. Peter Referral Hospital, Tikur Anbasa Specialized Hospital, Tirunesh Beijing General Hospital and, Zewditu Memorial Hospital. for granting me access to their facilities, their support was instrumental in carrying out this research effectively.

I am indebted to the members of EPHI for their guidance, support, and access to essential equipment and resources. Their expertise and assistance were pivotal in the completion of this thesis.

I am grateful to all the participants who volunteered and their willingness to participate in this research was essential to its success.

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Acronyms and abbreviations

AAU	Addis Ababa University
ANC	Antenatal Care
Anti-HBs	Antibody of Hepatitis B surface
CDC	Centers for Disease Control and Prevention
CHB	Chronic Hepatitis B
DNA	Deoxyribonucleic Acid
DRERC	Departmental Research and Ethics Review Committee
EPHI	Ethiopian Public Health Institute
HBcAg	Hepatitis Core Antigen
HBeAg	Hepatitis B e Antigen
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B Virus
MTC	Mother to Child
MTCT	Mother-to-Child Transmission
PCR	Polymerase Chain Reaction
rcDNA	Relaxed circular Deoxyribonucleic Acid
RTPCR	Real-Time Polymerase Chain Reaction
WHO	World Health Organization

Abstract:

Background: Hepatitis B virus infection is a significant global public health issue and is the leading cause of chronic hepatitis, which can ultimately lead to liver cirrhosis and liver cancer. This infection is mainly transmitted through blood and is often passed from mother to child. While there is a high prevalence of Hepatitis B Virus infection and mother-to-child transmission among delivering mothers in Ethiopia, there is limited evidence on the prevalence of mother-to-child transmission of Hepatitis B Virus and its risk factors in the country. Therefore, this study aimed to assess the mother-to-child transmission of the Hepatitis B Virus and its associated risk factors in Addis Ababa, Ethiopia.

Methods: A cohort study was carried out at selected government hospitals in Addis Ababa from April 2023 to May 2024 A total of 136 mothers and corresponding cord blood samples were collected and tested for Hepatitis B Virus viral load, by Abbott 2000 real-time, HBsAg, and HBeAg by Cobas e411 Socio-demographic characteristics and risk factor information were gathered through questionnaires. The data were analyzed using SPSS version 26, employing logistic regression analysis to identify factors associated with mother to child transmission of the Hepatitis B Virus. A significance level of 0.05 was considered.

Results: Out of 139 samples, 136(98%) HBV-positive mother-cord blood sample pairs were included. The mean ± SD age group was 27± 4.8 years. Most mothers (97.8%) resided in urban areas, and 46% had primary-level education. Among the mothers,100(73.5%) of mothers had detectable HBV DNA viral load, 12 (8.8%) were positive HBeAg, 90(66.2%) cord blood samples were HBsAg positive, 34(25%) had detectable HBV DNA viral load and 4(2.9%) of were positive for HBeAg. Among the study participants, 25(18.2%) of the mothers had a history of abortion, 30(21.9%) Female Genital Mutilation, and 5(3.6%) of mothers were living with known HBV-infected family members. Mother viral load (AOR) =0.184; 95% CI: 0.037-0.899; p-value (0.037) significant predictors of mother-to-child transmission of the Hepatitis B Virus.

Conclusion: In this study, the transmission rate was found (66.2%) and detectable viral load indicates a risk of vertical transmission. Other investigated potential transmission factors did not show significant associations with HBV infection,

Keywords: Hepatitis B virus, Mother-to-child transmission, Viral load, Associated Factor, Ethiopia.

1 Introduction

1.1 Background

Hepatitis B virus (HBV) is a global challenge and WHO estimates that 296 million people are diagnosed with chronic HBV infection and 686,000 people die each year from its complications, which include cirrhosis and hepatocellular carcinoma. In 2019, HBV was the cause of an estimated 820,000 deaths worldwide, and most deaths were from liver cirrhosis and primary hepatocellular carcinoma [1].

HBV is an enveloped partially double-stranded Deoxyribonucleic Acid (DNA) virus that belongs to the Hepadnaviridae family, in the Orthohepadnavirus genus, Its genome is a relaxed-circular DNA (rcDNA) with approximately 3,200 base pairs, with a complete minus strand and an incomplete plus strand [2].

HBV is a blood-borne infection and is mainly transmitted from mother to child, HBV causes acute as well as chronic infections [3]. Acute infection is usually self-limited and has a 0.5-1% case fatality rate. Chronic Hepatitis B (CHB) infection encompasses a spectrum of diseases and is the presence of detectable Hepatitis B Surface Antigen (HBsAg) in the blood or serum for longer than six months. HBsAg can be detected with or without active viral replication and with or without indications of hepatocellular damage and inflammation. Persons are considered uninfected if they are negative for both HBsAg and anti-Hepatitis B Core Antigen (HBc). Furthermore, anti-HBe positivity can be associated with the low replicative part of chronic hepatitis B virus [4].

Hepatitis B virus infection can be determined by diagnosing the presence of hepatitis B surface antibody (HBsAb), hepatitis B pre-core antigen (HBeAg), hepatitis B pre-core antibody (HBeAb), hepatitis B surface antigen (HBsAg), or hepatitis B core antibody (HBcAb) sero-marker reactivity. The presence of HBsAg represents active acute or chronic infections; whereas the HBeAg indicates high viral replication while HBsAb and HBeAb are indications of HBV resolution [5].

The major route of transmission of HBV is through Mother-To-Child Transmission (MTCT) which could be transplacental transmission of HBV in intrauterine transmission, natal transmission during delivery intrapartum transmission, and postnatal transmission during care postpartum transmission trans, Intrauterine transmission of HBV is indicated by the detection of either HBsAg or HBV DNA in

neonates born from HBV-infected mothers [6]. The rate of MTCT is higher in pregnant mothers with high HBV DNA viral load and HBeAg positives. Evidence indicated that MTCT can occur from mother viral load cut-off value 6 log10 copies/ml (5.3 log10 IU/ml) and above [6,7]. MTCT will occur in 70-90% of infants if the mother is HBeAg positive, the transmission will reduce to 40% when the mother is HBeAg negative [8]. Other evidence indicated that HBeAg-negative mothers have a near 0% risk of transmission, while HBeAg-positive mothers have a 20% risk of transmitting the virus [7,8].

In general, increase of transmission to 8-32% if the concentration of HBV DNA is high in the mother [8]. As a result, maternal high HBV DNA levels and HBeAg positivity in pregnant women are regarded as the most important risk factors for HBV MTCT [7,9] A study from Ethiopia indicated that 75% of newborns born from HBV-infected mothers were positive for HBsAg the rate of chronicity is much lower (<5%) among the adult population; however, it may reach 90% in infected newborns [10].

Approximately, 10% to 20% of HBsAg-positive pregnant women transmit HBV to their babies usually during birth or soon after birth. Mothers who were positive for both HBsAg and HBeAg have about a 100% chance of transmitting HBV to their newborns and over 85–90% of newborns infected at an early age will eventually become chronic carriers of the virus [15].

Therefore, the study aimed to assess Mother and child, HBeAg status (positive) and high HBV DNA viral load the association between HBeAg and HBV DNA viral load, and MTCT of HBV.

1.2 Statement of the Problem

According to the WHO research, the Western Pacific Region and the WHO African Region have the highest rates of chronic hepatitis B infection-116 million and 81 million persons, respectively. The WHO Eastern Mediterranean Region has sixty million infected individuals, the WHO South-East Asia Region has eighteen million, the WHO European Region has fourteen million, and the WHO Region of the Americas has five million. On the other hand, around one-third of persistent HBV infections worldwide are caused by MTCT of HBV during pregnancy or birth [7]. The amount of HBsAg and HBeAg in the mother's blood determines the transmission risk. For moms who tested positive for HBsAg and HBeAg, the transmission risk was 70–100% in Asia and about 40% in Africa [6].

Nearly half of HBV-infected patients have acquired the virus either through MTCT or during early infancy. Newborns infected with HBV through MTCT have an 85–90% chance of developing chronic HBV infection. These countries have moderate to high levels of HBV infection prevalence. Acute HBV infection in the first trimester of pregnancy carries a 10% risk of transmission to the fetus, but more than 60% of newborns may contract the infection in the period immediately before or during labor [23].

Sub-Saharan African nations also have a high prevalence of HBV infection, especially in those with underdeveloped healthcare systems. Pregnant women in Ethiopia have an HBV infection rate between 4.9% and 8.1%, which is moderate to high. This poses a serious risk to the health of the unborn child. Untreated mothers who carry the HBV virus run the risk of exposing their unborn child to the virus, mostly after childbirth [20]. Additionally, Ethiopian pregnant women do not regularly receive HBV testing and treatment, and the coverage is insufficient [22].

The impact of MTCT on HBV extends to both maternal and child health in Ethiopia. Children who receive MTCT and become chronically infected with HBV run the risk of developing liver illnesses such as cirrhosis and hepatocellular cancer. Low birth weight and preterm birth are two other negative consequences that can be caused by HBV infection during pregnancy. Improving the health of Ethiopian mothers and children requires addressing the prevention of MTCT of HBV [27].

Additionally, other studies have revealed an 11.6% prevalence of HBV infection among Ethiopian mothers. The rate of MTCT of HBV in the country stands at 30.9%. The issue of MTCT of HBV in

Ethiopia must be addressed immediately because of the high prevalence, low knowledge, gaps in prevention strategies, and issues with the healthcare system [31].

The main source of infection for children and the general public is the high incidence of HBV infection among pregnant women. Despite the high rate of HBV infection and MTCT among expectant mothers in Ethiopia, there is little information available about the prevalence of HBV MTCT and related risk factors. Therefore, it is imperative to conduct a thorough investigation on the MTCT of HBV and related variables in infants born to HBV mothers in Addis Ababa, Ethiopia.

1.3 Significance of the Study

This study identified the MTCT of the HBV and associated factors, The findings of this research have an impact on changing the country's health policies regarding the control and management of MTCT of the HBV.

By utilizing various serological markers and virological tests for HBV status, this study effectively determined the factors involved. Consequently, the findings were crucial for implementing and monitoring the appropriate utilization of prevention and control measures.

Furthermore, the finding serves as a valuable reference for future research conducted in this area. Since the study was conducted in 10 hospitals in Addis Ababa, which serve as referral centers for patients from different corners of the country, the results provided a good representation.

1.4 Literature review

1.4.1 Transmission and Prevalence of HBV infection in newborns from HBV-positive mothers

As per the information provided in the Hepatitis B Foundation fact sheet, the global HBV infection rate is about 30 million cases, 292 million chronic cases, 780,000 annual deaths, and one HBV-related death every 30 seconds [18]. According to a 2015 WHO report, 257 million people, or 3.5% of the world's population, have CHB infection; 68% of those cases were found in Africa and the Western Pacific region [7]. Furthermore, 5–10% of adults in East Asia and Sub-Saharan Africa have chronic infections [1].

The incidence of new HBV cases among the general population was estimated to be 10%–20% of the population each year by Khue P. *et al* cohort study on Hepatitis B Infection and MTCT in Vietnam in 2020. The study focused primarily on MTCT of HBV at birth (25.1%) and at 6 months show (13.1%) infants were HBsAg positive; the prevalence of positive HBsAg was high among infants born to HBeAg positive mothers and those who have a very high HBV DNA level [19]. According to the results of the national program on the prevention of MTCT of HBV, a cross-sectional study conducted in four representative provinces of China between October 2017 and January 2018 by Wang X. *et al* showed that the MTCT rate was 0.9% (0.6–1.1%) and that children born to mothers who tested positive for HBeAg had a higher MTCT rate than children born to mothers who tested negative for HBeAg [25].

According to a cross-sectional pilot study by Plymoth A. *et al* on hepatitis B mother-to-child transmission in Ghana's Eastern Region, the prevalence of MTCT was 5.9%, with three infants testing positive and 51 hepatitis B-infected women included in the study. Pregnancy-related viral marker testing was not available in the community and could not be evaluated with reliability [26]. Human immunodeficiency virus, hepatitis B, and hepatitis C virus MTCT rates in pregnant women in Nigeria range from 8.3% to 12.8%, according to a systematic review and meta-analysis by Uchenna G. *et al*. A recent study reveals that the pooled MTCT rate for HBV of 55.49% at birth is high and might not accurately reflect vertical transmission [27].

Reham R. *et al* study on hepatitis B and C prevalence in pregnant women in Egypt found a 12% vertical transmission rate, higher in rural areas (65%) than in urban areas (35%). Untrained midwives' use of non-sterile instruments also increased infection rates. From 2015 to 2016, 30.3% of pregnant women

had positive HBV cases [28]. Ethiopia's chronic HBV infection prevalence is 9.4%, with Afar having the highest prevalence at 28.8% and Harari at 4.9%, according to a Nationwide Seroprevalence in 2020 study by Atsbeha G [20]. A revised meta-analysis found a 5% pooled prevalence in expectant women by Yazie T *et.al* [13].

Alemu A. *et al.* conducted a systematic meta-analysis on HBV infection and its determinants among pregnant women from 2005 to 2020. The results showed that the pooled prevalence of HBV infection among pregnant women in Ethiopia was 4.75%, with a minimum of 2.3% in Southern Nations, Nationalities, and Peoples' Region and a maximum of 7.9% in Gambela [15]

A study conducted by Bayu H. *et al* in Arsi Zone Health Institutions, no Post-exposure prophylaxis coverage, vertical transmission, and associated factors among hepatitis B exposed newborns delivered in 2019, The vertical transmission rate found that 32.4% at birth, and even (26.7%) became hepatitis B surface antigen positive between 9^{th} and 12th months [29]. In this cross-sectional study on the seroprevalence of HBV infection, MTCT, and associated risk factors among delivering mothers in Tigray, by Kbrom K. *et al* The rate of MTCT of HBV infection shows 30.9% [23].

In 2014, Dessie T. *et al* did a cross-sectional study at St. Paul's Hospital Millennium Medical College and Selam Health Center in Addis Ababa, Ethiopia. The study indicated that the maternal positive rate for HBV surface antigen was low. Eight (3.0%) of the 265 study participants who underwent testing also tested positive for HBsAg, as did six (2.3%) of the child cord blood samples. Of the infant cord blood samples, six (75%) showed the same positive results as their infected mothers, while two (25%) showed negative results. Viral load testing was not done, however, only one case (12.5%) tested positive for HBeAg, another indicator of HBV infection [21]. Another cohort study on MTCT of HBV in Ethiopia by Desalegn H *et al* revealed 10.1% evidence of active HBV infection, either by a positive HBsAg result and/or detectable HBV DNA [30].

Teklu A. *et al* carried out another prospective cross-sectional study in 2018 at two hospitals in Addis Ababa, Ethiopia, to measure the rate of vertical HBV transmission and ascertain the seroprevalence of HBsAg among pregnant women. It was shown that 2.38% of research participants were positive for HBsAg. This prevalence rate belongs to the intermediate endemicity category. Overall, the results of

this study indicate that there was little chance of vertical HBV transmission in the community under investigation, as shown by the lack of HBsAg in cord blood samples [31].

1.4.2 Factors Associated with Hepatitis B virus infection

Mamuye B. *et al* conducted a cross-sectional study in the Eastern Hararghe part of Ethiopia from April to May 2017 on HBV infection and related factors among pregnant women attending antenatal clinics. The study found that the prevalence of HBV in pregnant mothers varies depending on the setting and is 6.1% among 363 pregnant women. Risk factors include having multiple sexual partners, having an abortion, having a traditional tonsillectomy, having been admitted to a health facility, and having a family member have had a liver disease [24].

According to a 2019 study on the seroprevalence of HBV infection, MTCT, and associated risk factors among delivering mothers carried out in Tigray by Kbrom K. *et al.* Body tattoos and hospital admission history were found to be significant risk factors for HBV infection in this cross-sectional study [23].

A study conducted on the Seroprevalence of Syphilis and HBV among Pregnant women at Saint Paul's Hospital Millennium Medical College, by Wabe Y. *et al.* from July to September 2019 showed that the Prevalence of 4.5% and among risk factors only multiple sexual partners was significantly associated with seroprevalence of HBV infection [10]. another cohort study conducted by Desalegn H *et al*. on MTCT of HBV in Ethiopia showed 10.1% evidence of active HBV infection, either by a positive HBsAg result and/or detectable HBV DNA, mothers with high HBV DNA viral load and HBeAg positive was a significant risk factor for HBV infected children [30].

Wabe Y. *et al* conducted a study on the seroprevalence of HBV infection and syphilis among pregnant women at Saint Paul's Hospital Millennium Medical College in 2019. The study found that the prevalence of HBV infection was 4.5%, and the only risk factor that was significantly associated with seroprevalence of HBV infection was having multiple sexual partners. Mothers with high HBV DNA viral load and HBeAg positive were a significant risk factor for HBV infected children [10, 29].

Furthermore, the WHO recommends using a reference line at 5.3 log10 IU/mL (equivalent to 200,000 IU/mL) as the threshold for a high risk of vertical transmission of the hepatitis B virus. In situations

where antenatal HBV DNA testing is unavailable, HBeAg testing can be used as an alternative to HBV DNA testing to determine eligibility for prophylaxis and prevent mother-to-child transmission of HBV [33].

Worldwide, HBV infection has a significant impact on public health. Nevertheless, little research on the MTCT of HBV has been done in Ethiopia. The results of the few published research conducted in Ethiopia on the transmission of HBV from mother to child have been conflicting. Numerous maternal characteristics, including as gestational age, mother age, and sociodemographic factors, have been investigated in this research. Maternal practices have also been taken into account, including a history of abortion and unprotected sex. The rate of HBV MTCT varies among populations and is impacted by several variables but it's crucial to take into account the mother's HBeAg status and the high levels of HBV DNA on HBV transmission.

2 Objectives

2.1 General Objective

To assess the mother-to-child transmission of the Hepatitis B Virus and associated factors in Addis Ababa, Ethiopia, 2023-2024.

2.2 Specific Objectives

To assess the transmission rate of HBV infection to newborns from HBV-positive mothers.

To identify the factors associated with mother-to-newborn transmission of hepatitis B virus.

3 Materials and Methods

3.1 Study area

This study was conducted in Addis Ababa, the capital city of Ethiopia. It is the largest city in the country with an estimated area of 530.14 km^2 and a population density of 5,607.96 individuals per km^2. It has an estimated total population of 3.5 million in 2017 based on 2007–2037 population projection [32].

Government hospitals that provide antenatal care (ANC) and delivery services at Addis Ababa. Those are Abebech Gobena Hospital, Alert Hospital, Federal Police Hospital, Dagmawi Minilik II Hospital, Gandi Memorial Hospital, Ras Desta Damtew Memorial Hospital, St, Paul Hospital Millennium Medical College, St. Peter Referral Hospital, Tikur Anbasa Specialized Hospital, and Zewditu Memorial Hospital.

3.2 Study Design and Period

A Cohort study was conducted to assess the MTCT of HBV and Associated Factors from April 2023 to May 2024 G.C.

3.3 Population

3.3.1 Source population.

The source population was all pregnant mothers and infants born from those mothers at government Hospitals in Addis Ababa, Ethiopia, from April 2023 to May 2024 G.C.

3.3.2 Study population.

The study population was HBV-infected pregnant mothers and infants born from HBV-infected mothers at government Hospitals in Addis Ababa, from April 2023 to May 2024 G.C.

3.4 Inclusion and Exclusion Criteria

3.4.1 Inclusion criteria

All HBV-positive mothers attending antenatal care during the study period in governmental hospitals in Addis Ababa and who gave their consent were included in the study.

All infants born from HBV-positive mothers during the study period whose mothers signed guardian assent forms were included in this study.

3.4.2 Exclusion criteria

HBV-positive mothers who interrupted ANC follow-up and who didn't give birth at the hospital were excluded.

HBV-positive mothers who didn't give their consent and assent were excluded from the study.

3.5 Study Variables

3.5.1 Dependent variables

HBV Status of Infants born from infected mothers.

3.5.2 Independent variables

Socio-demographic characteristics: age, educational level, occupation, marital status, history of abortion, circumcision, and viral load.

3.6 Measurement and Data Collection

3.6.1 Sample Size Determination

The sample size for this was calculated by using a single proportion formula using 10.1% true population prevalence HBV exposed infants from previous studies [20], 0.05 levels of precision, and a 95% confidence level.

The value of p was taken as 10.1 % (0.101).

Where n = sample size, Z = Z statistic for a level of confidence, P = expected prevalence or proportion (P = 0.101), and d = precision (d = 0.05), Z = Z statistic: For the level of confidence of 95%, which is conventional, Z value is 1.96.

Therefore, the sample $n = \frac{z\alpha/2\, p*(1-p)}{d^2}$

$n = [1.96 \times 0.1(1-0.1)]/0.05^2$

$n = 139$

3.6.2 Sampling method

A Purposive sampling technique was employed to include all HBsAg-positive mothers' blood during the third trimester and cord blood of Infants born from HBsAg-positive mothers in the Hospitals, to select sample size from the hospital's proportional sample allocations were done for each hospital, from their previous year's report (2022 G.C) of HBsAg positive ANC follow up.

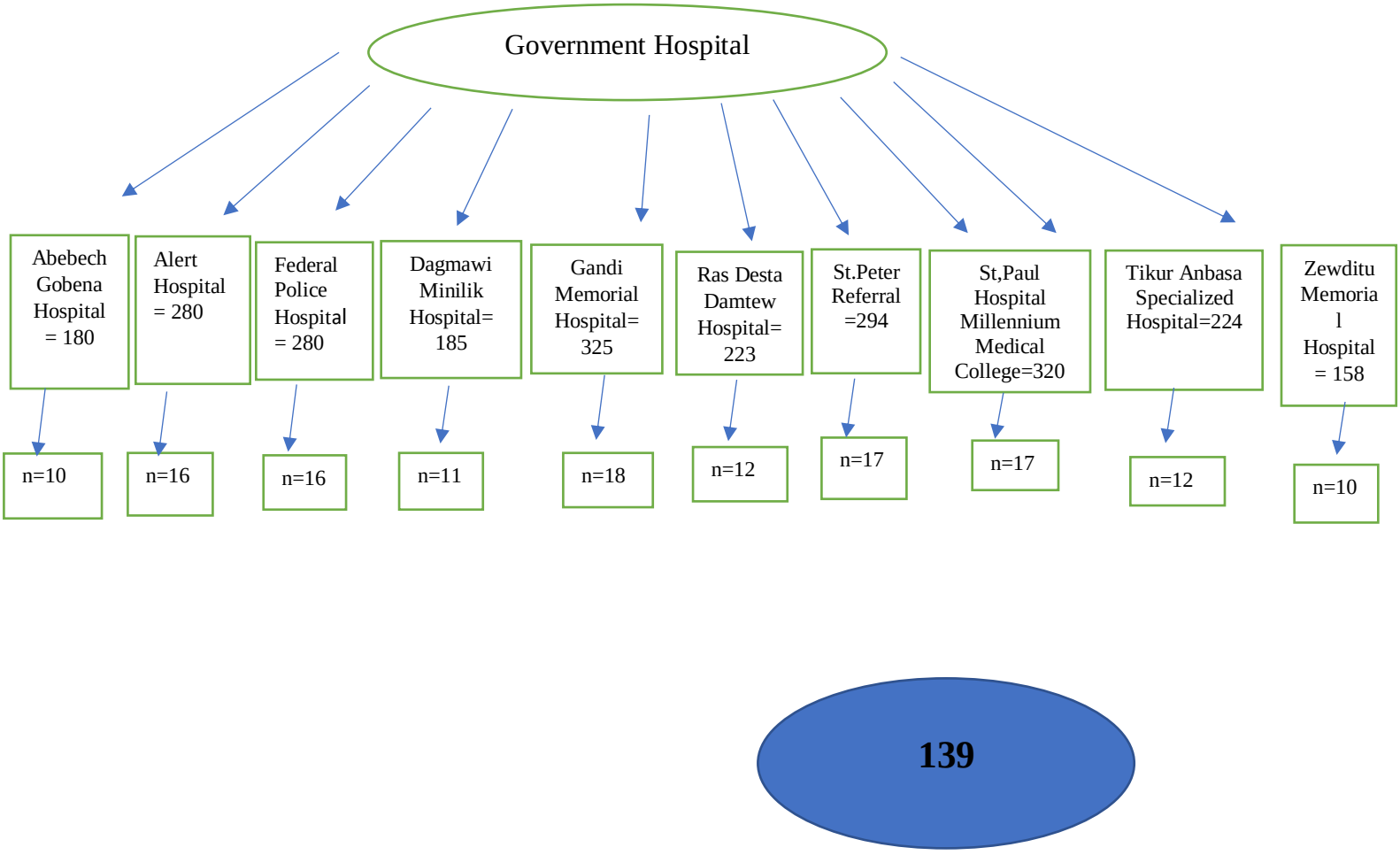

Figure 2: Proportional sample allocations for the mother-to-child transmission of the Hepatitis B Virus and associated factors in Addis Ababa, Ethiopia,2024.

3.6.3 Data collection procedure

Data on the Socio-demographic characteristics of the mothers were collected through Structured, questionnaires to assess other associated risk factors. **(Annex I).** Data were collected by using ODK Collect, Open Data Kit (ODK). ODK is an open-source suite of tools that allows data collection using Android mobile devices and data submission to an online server. It replaces traditional paper questionaries with electronic forms that allow text, numeric data, GPS, photo, video, barcodes, and audio uploads to an online server.

ODK tool kits offer features and support that can help organizations (investigators) manage ethical issues related to data security, user control, community involvement, and compliance. We have captured photo signatures of study participants during data collection and provided an Excel of data collection columns as Yes if the participants were willing to participate.

From all HBsAg-positive pregnant mothers, a 5 ml whole blood sample was collected at 26-28 weeks of pregnancy or as early as possible then the collected samples were transported to the Ethiopian Public Health Institute (EPHI) for laboratory analysis. After Plasma was separated from the sample an HBsAg test was performed to confirm before inclusion in the study. In addition, for infants born from those mothers, 5 ml cord blood was collected immediately after birth within 24 hours.

3.6.4 Principles of each Laboratory analysis

This Cohort study design was conducted on MTCT of Hepatitis B Virus and Associated Factors in a serological and virological study, all mothers' status of HBV infection was confirmed and to see the associated factors of MTCT of HBV HBsAg positive pregnant mothers were tested for HBeAg test to see an active viral replication by using Electrochemiluminescence Assay (Elecsys) Cobas e 411 immunoassay analyzers machine based on manufacturer instruction. **(Annex III).** and HBV DNA test to see the viral load concentration by real-time polymerase chain reaction (RT-PCR) by Abbott m2000sp/rt machine based on manufacturer instruction. **(Annex VI).**

Furthermore, the infant infection status was determined by HBV marker tests such as HBsAg and HBeAg using a Cobas e 411 immunoassay analyzer machine. in addition, HBV DNA was tested by Abbott m2000sp/rt machine.

The HBsAg test is used to detect the presence of the hepatitis B surface antigen (HBsAg) in a patient's blood sample. HBsAg is a marker of HBV infection.

The HBsAg test is primarily used for the diagnosis and screening of hepatitis B infection. It helps identify individuals currently infected with HBV, including acute and chronic infections. The test is also used to monitor the effectiveness of treatment and to screen individuals at risk of transmitting the virus. If the measured signal falls below the predefined cutoff value, the result is reported as negative. This indicates that HBsAg is not detected in the sample, suggesting the absence of current HBV infection.

The measured signal exceeds the cutoff value, ≥1.0 the result is reported as positive. This indicates the presence of HBsAg in the sample, suggesting current HBV infection. Further testing may be required to determine if the infection is acute or chronic.

The HBeAg test is used to detect the HBeAg in a patient's blood sample. The HBeAg is a marker of active viral replication in HBV infection.

The test is primarily used to assess the replication activity of the HBV and to determine the infectiousness of an individual with chronic hepatitis B. It is commonly performed in conjunction with other hepatitis B tests to provide a comprehensive evaluation of the infection status.

Negative Result is the measured signal falls below the defined cutoff value of <1.0 the result is reported as negative. This suggests the absence of HBeAg in the sample, indicating lower viral replication activity and potentially lower infectivity.

Positive Result is the measured signal exceeds the cutoff value of ≥ 1.0 the result is reported as positive. This indicates the presence of HBeAg in the sample, suggesting active viral replication and higher infectivity.

The HBV DNA test is used to detect and quantify the amount of hepatitis B virus (HBV) DNA in a patient's blood. It helps in assessing the replication level of the virus. The test is performed to determine the viral load, or the amount of HBV DNA present in the blood. It is used to assess the severity of the infection, monitor the effectiveness of antiviral treatment, and detect viral replication activity. The test is particularly important in identifying individuals with chronic hepatitis B.

Lower Viral Load: A lower viral load indicates a lower amount of HBV DNA in the blood. It may suggest a less active or controlled infection and a potentially lower risk of disease progression.

Higher Viral Load: A higher viral load indicates a higher amount of HBV DNA in the blood. It may suggest a more active or uncontrolled infection and a potentially higher risk of disease progression.

3.7 Data Quality Assurance:

ANC and deliver unit nurses reviewed the registration book of mother-infant pairs and collected factors associated with transmission by a predesigned questionnaire after prior training on the objectives of the study and data quality. The questionnaire was tested before use In St. Paul's Hospital Millennium Medical College.

The principal investigator regularly supervises the data collection procedure and corrects any data collection errors as soon as possible. Laboratory personnel and midwives also get training in blood collection. Standard Operating procedures were prepared for every laboratory procedure and every procedure performed by competent laboratory personnel.

3.8 Data analysis and interpretation

Data was checked and cleaned for any error before being analyzed, then the main analysis was carried out using SPSS version 26. In the descriptive analysis, both central tendency and dispersion were presented using frequency tables while the inferential statistics were carried out by using binary logistic regression. Values that have a p-value < 0.2 in the bivariate analysis were further analyzed in a multivariable analysis. An adjusted Odds Ratio with a 95% confidence interval was reported for those variables with a p-value < 0.05 in the multivariable analysis to establish an association with MTCT of hepatitis B infection.

3.9 Ethical considerations

Before starting the research work, ethical clearance was obtained (Ref. No. MLS/161/23) from the Departmental Research and Ethics Review Committee (DRERC) of the School of Medical Laboratory Sciences, Addis Ababa University. Written informed consent for the mothers and parental consent was obtained from the mothers of HBV-exposed infants after thoroughly understanding the objective of the study. Any information that exposes the identity of an individual was removed and replaced with codes. Data access was limited to the data manager and the principal investigator through a password for electronic files and a locked cupboard for hard copies.

3.10 Operational Definitions:

Hepatitis B surface antigen (HBsAg): - is a viral protein found on the surface of hepatitis B virus it is an important marker used in diagnosing HBV and detected in high levels in serum during acute or chronic hepatitis B virus infection.

Mother to Child Transmission of Hepatitis B Virus: considered present if the newborn cord blood is positive for Hepatitis B surface antigen (HBsAg).

Viral Load: Quantitative measurement of Hepatitis B viral DNA in maternal blood.

4.. Workflow

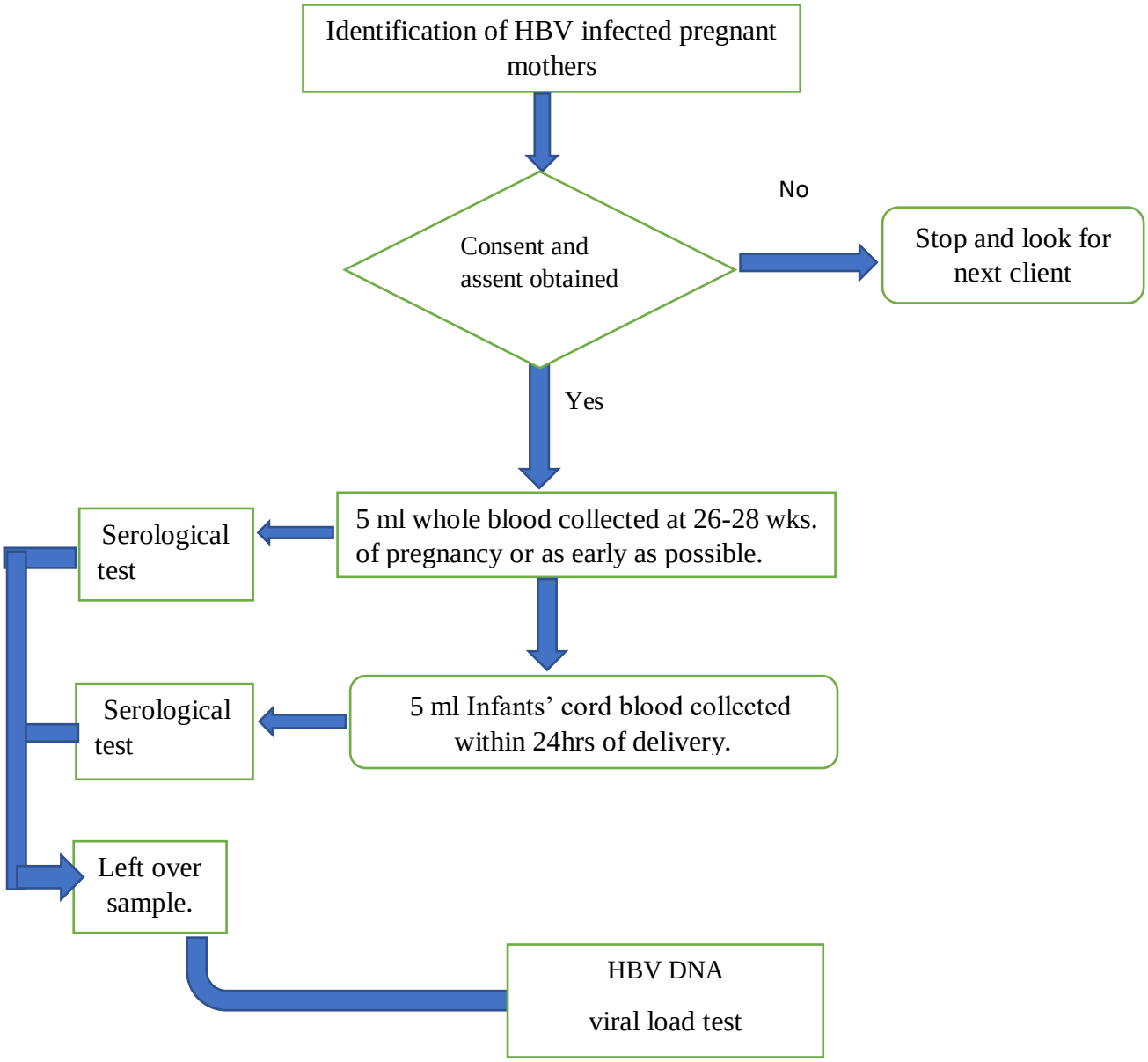

Figure 2: Specimen and data collection flow chart of the mother-to-child transmission of the Hepatitis B Virus and associated factors in Addis Ababa, Ethiopia,2024.

5. Results

5.1 Socio-demographic characteristics of the study participants

From the calculated 139 sample size, 136(98%) of HBsAg-positive pregnant women were involved in this study. The mean ± SD age group was 27± 4.8 years. The lowest age was 19 years, and the maximum age was 40 yrs. About 133(97.8%) of pregnant women live in urban areas and the remaining 3(2.2%) them are from rural areas on the outskirts of Addis Ababa. Most pregnant women were married. [Table 1].

Table 1. Summary of socio-demographic characteristics of the study participants in mother-to-child transmission of the Hepatitis B Virus and associated factors in Addis Ababa, Ethiopia,2024

Variables	Number (N)	Percent (%)
Age (Years)		
18-25	56	41%
26-35	68	50%
36-45	12	8.8%
Residence		
Urban	133	97.8%
Rural	3	2.2%
Educational levels of the mother		
No formal education	3	2.2%
Primary	63	46%
Secondary	35	25.5%
Tertiary	35	25.5%
Occupation of the mothers		
Employee	28	20.4%
Housewife	88	64.2%
Merchant	18	13.1%
Student	2	1.5%
Marital status		
Married	132	98.4%
Unmarried	2	1.5%
Divorced	2	1.5 %

5.2 Serological and virological HBV infection status of newborns from HBV-positive mothers

From total HBV Positive mothers included in the study 3 mothers had their HBsAg confirmatory tests turn negative that leading to exclusion So, 136 participants consented to be followed and were seen at their delivery visits for the collection of cord blood samples. Laboratory tests for HBsAg and HBeAg were performed by using the Electrochemiluminescence Assay (Elecsys) Cobas e 411 immunoassay analyzer machine. HBV DNA viral load testing was conducted for both the mothers and cord samples using real-time PCR (polymerase chain reaction) with the Abbott m2000sp/rt.

Out of those participant mothers, 12 (8.8%) were positive HBeAg, and 124(90.5%) HBeAg negative. While out the corresponding 136 Cord blood samples, 90(66.2%) were positive for HBsAg and 4(2.9%) were positive for HBeAg [Table 2].

Table 2. Serological markers for pregnant mother and cord blood sample among participants in mother-to-child transmission of the Hepatitis B Virus and associated factors in Addis Ababa, Ethiopia,2024

HBeAg status of the mother		Serological markers of cord blood sample			
HBeAg Negative N (%)	HBeAg Positive N (%)	HBsAg Positive N (%)	HBsAg Negative N (%)	HBeAg Positive N (%)	HBeAg Negative N (%)
124(90.5%)	12(8.8%)	90(66.2%)	46(33.8%)	4(2.9%)	132(97.2%)

The other laboratory test performed was the quantification of HBV DNA viral load. The result showed that 101(74.2%) mothers had detectable HBV DNA viral load. From the detectable viral load, 16 (11.7%) of mothers had >200,000 IU/mL HBV DNA viral load. Among the HBsAg confirmed mothers 35(25.7%) of them had undetected HBV DNA viral load.

Out of the 90(66.2%) HBsAg-positive cord blood samples, 34(25%) had detectable HBV DNA viral load. 3(2.2%) of them having >200,000IU/ML HBV DNA Viral load. The remaining 102(75%) samples had undetectable HBV DNA [Table 3].

Table 3. Summary of HBV DNA viral load for pregnant mothers and Cord blood sample among participants in mother to child transmission of the Hepatitis B Virus and associated factors in Addis Ababa, Ethiopia,2024

HBV DNA Viral Load	Pregnant women HBV DNA Viral Load N (%)	Cord blood HBV DNA Viral Load N (%)
>200,000IU/ML	16(11.7%)	3(2.2%)
≤200,000IU/ML	85(62.5%)	31(22.8%)
Undetectable	35(25.7 %)	102(75%)

5.3 Associated Factors of Mother to Newborn Transmission of Hepatitis B virus among Newborns in Addis Ababa, Ethiopia

Out of 136 HBsAg-positive pregnant mothers, 25(18.2%) of the mothers had a history of abortion 30(21.9%) had Female Genital Mutilation (circumcision), and 65(47.4%) of the mothers had known HBV infection status before pregnancy. Only 4(2.9%) mothers were vaccinated against HBV and 5(3.6%) mothers were living with known HBV-infected family members. We also conducted binary logistic regression to investigate other potential factors associated with MTCT of HBV infection and those variables.

In this study, among factors analyzed in binary logistic regression, four of them had Values of less than (p-value < 0.2) were potential confounders further analyzed using Multivariable analysis, we found that Mother HBV DNA viral load >200000 IU/ml (Adjusted odds ratio (AOR) =0.184; 95% CI: 0.037-0.899; p-value (0.037) was significantly associated with MTCT of HBV infection after adjusting for potential confounders [Table 4].

Table 4. Bivariate and Multivariate logistic regression showing factors associated with HBV infection among pregnant women of the study participants in mother to child transmission of the Hepatitis B Virus and associated factors in Addis Ababa, Ethiopia,2024.

Variables	Cord HBs Ag positive	Cord HBsAg Negative	Bivariate analysis COR (95 % CI)	*p-value*	Multivariate analysis AOR (95 % CI)	*p-value*
Age group						
18-25 Yrs.	41	27	1			
26-35 Yrs.	8	4	1.250(0.330-4.740)	0.743		
36-45 Yrs.	40	16	0.759(0.208-2.772)	0.677		
Educational levels of the mother						
No formal education	1	2	0.375(0.31-4.532	0.440	0.442 (0.034-5.724)	0.532
Primary	20	15	1.737(0.736-4.100)	0.199	1.65 (0.645-4.224)	0.296
Secondary	24	11	1.636(0.615-4.353	0.324	2.019 (0.706-5.772)	0.190
Tertiary	44	19	1			
History of Circumcision						
Yes	69	37	0.932(0.395-2.199)	0.873		
No	20	10	1			
History of abortion						
Yes	72	39	0.869(0.344-2.194	0.766		
No	17	8	1			
Known HBV infection before pregnancy						
Yes	38	27	1			
No	51	20	1.812(0.887-3.703	0.103	1.632 (0.761-3.502)	0.208
Mothers being vaccinated against HBV						
Yes	1	3	1			
No	88	44	6.00(0.606-59.236)	0.125	4.046 (0.356-45.9)	0.260
Number of pregnancies						
1	5	9	1			
2	25	15	1.667(0.097-28.66)	0.725		
≥ 3	59	23	3.000(0.173-52.10)	0.451		
Having family members infected with HBV						
Yes		47	0.462(0.050-4.255	0.495		
No		89	1			
Mother's viral load concentration						

Undetectable	23	12	1			
(HBV DNA <200,000 IU/mL)	52	33	0.274(0.053-1.408)		0.273 (0.051-1.467)	0.130
(HBV DNA >200,000 IU/mL)	14	2	0.225(0.048-1.055)	0.058	0.184 (0.037-0.899)	0.037

6. Discussion

Identifying and quantifying the transmission of Hepatitis B Virus from mother to child and identifying the associated factors in the Government Hospital in Addis Ababa, Ethiopia, using different laboratory testing mechanisms, were very crucial for diagnosing and monitoring HBV infection as Mother to child transmission is the major route of HBV infection.

In this study, the mother-to-child Transmission (MTCT) rate of HBV was found to be 66.2%. This finding aligns closely with a pooled transmission rate reported from Nigeria, which was 55.49% [28]. However, the transmission rate observed in this study is notably higher than rates reported in studies conducted in various regions such as China (0.9%) [25], Egypt (12%) [26], Ghana (5.9%) [27], Arsi Ethiopia (32.4%) [29], Tigray Ethiopia (30.9%) [23], and Addis Ababa, Ethiopia (10.1%) [20].

These discrepancies in transmission rates could be attributed to the geographical context of the study population and the varying preventive measures implemented across different countries, particularly outside of Ethiopian studies. Even within Ethiopia, differences might stem from the methodologies employed A relatively higher prevalence of MTCT HBV is observed in studies conducted within Ethiopia, specifically in Arsi Ethiopia (32.4%) [29] and Tigray northern Ethiopia [23]. Our study was a cohort study, whereas others were cross-sectional. Notably, the MTCT HBV rate identified in our study is lower than the rate reported in a previous study in Addis Ababa, Ethiopia (75%) [30]. This variance could be due to the time gap between the two studies and the difference in sample sizes used. Our study included 136 participants, whereas studies conducted by Tegegne D. et al. in 2014 had only 7 participants.

This study suggests that a high viral load concentration in mothers may be an important factor associated with the transmission or severity of HBV infection to their offspring. Mother HBV DNA viral load >200,000 IU/ml, is significantly related to vertical transmission of HBV to newborns. Newborns born from mothers with > 200,000 IU/ml viral load were at an 18.4 times higher risk compared with newborns born from mothers with < 200,000 IU/ml. positive for HBsAg which shows among Mother HBV DNA viral load.

The rate of MTCT is higher in pregnant mothers with >200,000 IU/ml HBV DNA viral load and HBeAg positives. This finding correlated with study findings in China [25]. Vietnam [19], Ethiopia

[29]. Other factors assessed by this study did not have a statistically significant finding. However, it is important to note that this does not necessarily indicate the absence of a relationship between the factors and MTCT HBV.

Generally, MTCT of HBV infection is still high in Ethiopia. This might be due to inadequate treatment for HBsAg carrier mothers and inadequate vaccination coverage for pregnant mothers. Therefore, preventing MTCT is essential to achieving the WHO goal of HBV elimination by 2030 [34]. This can be achieved through the antiviral treatment of HBeAg-positive pregnant women and birth dose vaccination for newborns from HBsAg carrier mothers [35]. However, it is not yet implemented in Ethiopia [36]. Therefore, early recognition and treatment of HBV-carrier pregnant women and the introduction of birth dose vaccination for newborns from HBsAg-positive mothers will reduce MTCT in Ethiopia.

7. Conclusions and Recommendations

7.1 Conclusions

In this study, the mother-to-child Transmission (MTCT) rate of HBV was 66.2%. This study found that there were a significant number of newborn cord blood positive for HBV serological test and a few of them with active replication. Mothers' high viral load concentration was found to be a factor in MTCT of HBV.

7.2 Recommendations

It's important to monitor pregnant women with HBV for viral replication markers such as HBeAg and HBV DNA levels, as these factors can increase the risk of passing the infection to their newborn. Mothers with indications of viral replication and high viral load should be treated to suppress the viral replication and prevent MTCT of HBV. In addition, it is crucial to provide newborns born from HBV mothers an immunoprophylaxis and vaccine at birth.

8. Strengths and Limitations of the Study

The study design is a cohort study following a participant, collecting a large number of Cord blood samples and serological and virological laboratory analysis are some of the strengths of this study. Loss to follow-up and conducting the study in an urban area are the limitation of the study. Therefore, the findings may not be generalizable to the national situation. Indeed, the sample consisted of women living in one of the largest urban areas of Addis Ababa, Ethiopia.

9 References

1. World Health Organization. Global Health Sector Strategy on Viral Hepatitis 2021. WHO. 2021;232.
2. Tsukuda S, Watashi K. Hepatitis B virus biology and life cycle. Antiviral Res. 2020; 182:104-925
3. World Health Organization. Preventing Perinatal Hepatitis B Virus Transmission: A Guide for Introducing and Strengthening Hepatitis B Birth Dose Vaccination. 2015:1–12.
4. World Health Organization. Guidelines for the prevention, care, and treatment of persons with chronic hepatitis B infection. Geneva, Switzerland: WHO; 2015.
5. Guo Z. Shi X. Feng Y. Wang B. Feng L. Wang S. et al. Risk factors of HBV intrauterine transmission among HBsAg- positive pregnant women. J Viral Hepat. 2014; 20:317–321.
6. Yi P. Chen R. Huang Y. Zhou R. and Fan X. Management of mother-to-child transmission of hepatitis B virus: Propositions and challenges. J. Clin. Virol. 2016;77: 32–39.
7. World Health Organization. (2017). Global hepatitis report. 2017.
8. Whittaker G. and Herrera L. Hepatitis B in pregnancy. South. Med. J. 2014; 107:195–200.
9. Al-Amleh S. Prevalence of hepatitis B virus among children of HBsAg-positive mothers in Hebron district, Palestine. Transl. Gastroenterol. Hepatol.2020;5:8–13.
10. Wabe Y. Reda D. and Ali M. "Seroprevalence of Syphilis and HBV among Pregnant Women at Saint Pauls Hospital Millennium Medical College, Addis Ababa Ethiopia." Prim. Heal. Care. 2021; 11:1–6.
11. Babatunde O. Smuts H. Eley B. Korsman S. de Lacy R. and Hardie D. Fulminant R. hepatitis B virus (HBV) infection in an infant following mother-to-child transmission of an e-minus HBV mutant: Time to relook at HBV prophylaxis in South African infants. South African Med. J. 2018; 108:389–392.
12. Chen X, Chen J, Wen J, Xu C, Zhang S, Zhou YH, et al. Breastfeeding is not a risk factor for Mother-to-Child Transmission of the Hepatitis B Virus. PLoS ONE. 2013;8: 55-303.
13. Yazie T and Tebeje M. An updated systematic review and meta-analysis of the prevalence of hepatitis B virus in Ethiopia. BMC Infect. Dis.2019; 19:1–13.
14. Osiowy C. Sensitive detection of HBsAg mutants by a gap ligase chain reaction assay. J. Clin. Microbiol. 2002;40: 2566–2571.

15. Alemu A, Zeleke L, Aynalem B, and Kassa G. Hepatitis B Virus Infection and Its Determinants among Pregnant Women in Ethiopia: A systematic review and meta-analysis. PLoS One. 2020; 15:10
16. Komlos J and Landes R. Prevention of Hepatitis B Virus Infection in the United States: Recommendations of the Advisory Committee on Immunization Practices. Econ. Hist. Rev. 1991; 44:133–136.
17. Chen D. S. Hepatitis B vaccination: The key towards elimination and eradication of hepatitis B. *J. Hepatol.*2009;50:805–816.
18. HBV. Foundation, "Hepatitis B Fast Facts," 2018. date accessed 26,12,2021.
19. Khue P. M, Thuy Linh N. T, Vinh V. H, Dung L. V, and Nguyen Van B. Hepatitis B Infection and Mother-to-Child Transmission in Haiphong, Vietnam: A Cohort Study with Implications for Interventions. Biomed Res. Int.2020; 20:1-9.
20. Weldemariam A. "Nationwide Seroprevalence of Hepatitis B Virus Infection in Ethiopia: A Population-Based cross-sectional Study," J. Infect. Dis. Diagnosis Abstr. 2020.
21. Tegegne D. Desta K. Tegbaru B. and Tilahun T. Seroprevalence and transmission of Hepatitis B virus among delivering women and their newborn in selected health facilities, Addis Ababa, Ethiopia. BMC Research Notes 2014; 7: 1-4
22. Navabakhsh B. Mehrabi N. Estakhri A. and Mohamadnejad M. Hepatitis B Virus Infection during Pregnancy: Transmission and Prevention. Middle East Journal of Digestive Diseases. 2011; 3:92–102.
23. Kbrom K. Mekonnen G. Yohannes T. et al Seroprevalence of Hepatitis B Virus Infection, Mother- To-Child Transmission, and Associated Risk Factors Among Delivering Mothers in Tigray Region, Northern Ethiopia: A Cross-Sectional Study. Infect. Dis. 2020; 9:901–911.
24. Mamuye B, Gobena T, Oljira L. Hepatitis B virus infection and associated factors among pregnant women attending antenatal clinics in west Hararghe public hospitals, Oromia region, Ethiopia. Pan Afr. Med. J. 2020; 35:1–9.
25. Qiao Y. Su M. Song Y. Wang X. et.al Outcomes of the national program on prevention of mother-to-child transmission of hepatitis B virus in China, 2016–2017. Infectious Diseases of Poverty. 2019; 8:65.
26. Hambridge T. Nartey Y. Duah A.Plymoth A..al Hepatitis B mother-to-child transmission in the Eastern Region of Ghana: a cross-sectional pilot study. Pan African Medical Journal. 2019; 33:218.

27. Uchenna G. Ukamaka C. Owen P. Innocent I. et.al Mother-to-child transmission of human immunodeficiency virus, hepatitis B virus, and hepatitis C virus among pregnant women with single, dual, or triplex infections of human immunodeficiency virus, hepatitis B virus and hepatitis C virus in Nigeria: A systematic review and meta-analysis SAGE Open Medicine 2022; 10: 1–15.
28. Reham R. Hany H. Prevalence of hepatitis B and C in pregnant ladies and their neonates in Minia governorate Int J Pregn & Chi Birth. 2018; 4:71–73
29. Bayu H. Elias B. Abdisa S. Tune A. Post exposure prophylaxis coverage, vertical transmission and associated factors among hepatitis B exposed newborns delivered at Arsi zone health institutions, PLoS One 2019; 15:10
30. Johannessen A, Mekasha B, Desalegn H, Aberra H, Stene-Johansen K, Berhe N. Mother-to-Child Transmission of Hepatitis B Virus in Ethiopia. Vaccines. 2021; 9:430.
31. Teklu A. Desta K. Tsegaye A. Sero-prevalence of HBsAg among Pregnant Women and Rate of Vertical Transmission in Selected Health Facilities of Addis Ababa, Ethiopia. Addis Abeba University Library, 2021;07:2-5
32. Ethiopian Central Statistical Agency, "Population Projections for Ethiopia: 2007-2037," 2013.
33. World Health Organization. Prevention of mother-to-child transmission of hepatitis B virus: guidelines on antiviral prophylaxis in pregnancy. Policy brief, 2020
34. Jourdain G, Ngo-Giang-Huong N, Khamduang W. Current progress in the prevention of mother-to-child transmission of hepatitis B and resulting clinical and programmatic implications. Infect Drug Resist. 2019; 12:10-19.
35. Sheng Q-J, et al. Hepatitis B virus serosurvey and awareness of mother-to-child transmission among pregnant women in Shenyang, China: an observational study. Medicine (Baltimore). 2018; 97: 1-09
36. Amsalu A, et al. Prevalence, infectivity, and associated risk factors of hepatitis B virus among pregnant women in Yirgalem Hospital, Ethiopia: implication of screening to control mother-to-child transmission. J Pregnancy. 2018; 84:35-91.

Annexes

Annex I: Questionnaires (English Version)

1. Age----------
2. Highest educational levels of the mother
   A. No formal education
   B. Primary
   C. Secondary
   D. Tertiary
3. Marital status
   A. Married
   B. Unmarried
   C. Divorced
   D. Widowed
4. Employment status of the mothers
   A. Student
   B. Employee
   C. Housewife
   D. Merchant
5. Residence
   A. Urban
   B. Rural
6. Mothers being vaccinated against HBV
   A. Yes
   B. No
7. History of Female Genital Mutilation (Circumcision) of the mother
   A. Yes
   B. No
8. History of abortion
   A. Yes
   B. No
9. Number of pregnancies

A. 1

B. 2

C. ≥ 3

10. HBV infection is known before pregnancy.

A. Yes

B. No

11. Having family members infected with HBV.

A. Yes

B. No

Annex II: የአማርኛ መጠይቅ

1. እድሜ----------
2. የትምህርት ደረጃ

   ሀ. መደበኛ ትምህርት ያለተማ

   ለ. የአንደኛ ደረጃ ትምህርት

   ሐ. ሁለተኛ ደረጃ ትምህርት

   መ. የኮሌጅ ወይም ዩኒቨርሲቲ ትምህርት የተከታተልኩ

3. የትዳር ሁኔታ

   ሀ. ያገባች

   ለ. ያላገባች

   ሐ. የፋታች

   መ. ባል የሞተባት

4. የስራ ሁኔታ

   ሀ. ገበሬ

   ለ. ሰራተኛ

   ሐ. የቤት እመቤት

   መ. ነጋዴ

   ሰ. ተማሪ

5. የመኖሪያ አድራሻ

   ሀ. ከተማ

   ለ. ገጠር

6. እናት የ HBV ክትባት ተከትበዋል
   ሀ. አዎ ተከትቢያለሁ

   ለ. አልተከተቡም

7. የእናት የግርዛት ሁኔታ

   ሀ. የተገረዙ

   ለ. ያልተገረዙ

8. ከዚህ ቀደም ውርጃ አጋጥሞት ያውቃል

    ሀ. አዎ ያውቃል

    ለ. አጋጥሞኝ አያውቅም

9. ይህ እርግዝና ስንተኛ እርግዝናዎ ነው

    ሀ. 1

    ለ. 2

    ሐ. ≥3

10. ከእርግዝናዎ በፊት HBV በሽታ እንዳለቦት ያውቅ ኖሮዋል

    ሀ. አዎ

    ለ. አላውቅም

11. ከእርስዎ ሌላ ከቫይረሱ ጋር የሚኖር ሌላ የቤተሰብ አባል አለ

    ሀ. አዎ አለ

    ለ. የለም

Annex III: Principal of Cobas e 411 assay

The Elecsys assay was an electrochemiluminescence microparticle assay, which measured the concentration of the antibody–antigen complexes in a similar manner to the Architect assays.
The Cobas e 411 assay platform provided an automatic dilution function, the test duration of the assay was 18-25 minutes. The analytic measurement range proposed by the manufacturer was between 5 IU/mL and 13,000 IU/mL, in which the samples were diluted 100-fold using the automated dilution function, and the Results were determined automatically by the software in a total duration of 18-20 minutes essay by comparing the electrochemiluminescence signal obtained from the reaction product of the sample with the signal of the cutoff value previously obtained by calibration.

A) HBsAg, Test principal

Specimen collection and preparation

Blood was collected using standard sampling tubes of heparin, K2-EDTA, K3-EDTA, ACD, CPD, CP2D, CPDA, and Na-citrate plasma as well as plasma tubes containing separating gel. Centrifuge samples containing precipitates before performing the assay.
Serum Samples Stable for 5 days at 2-8 °for 3 months at -20 °C (± 5 °C).

Reagents - working solutions.

The reagent rack pack (M, R1, R2) is labeled as HBSAG II.
M Streptavidin-coated microparticles
R1 Anti-HBsAg-Ab~ biotinylated monoclonal anti-HBsAg antibodies
R2 Anti-HBsAg-Ab- Monoclonal anti-HBsAg antibody, polyclonal anti-HBsAg

Test principle

Sandwich principle. Total duration of assay: 18 minutes.
▪ 1st incubation: 50 µL of sample, two biotinylated monoclonal anti-HBsAg antibodies, and a mixture of monoclonal anti-HBsAg antibody and polyclonal anti-HBsAg antibodies labeled with a ruthenium complexa) form a sandwich complex.
▪ 2nd incubation: After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.
▪ The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode.

Unbound substances are then removed with ProCell / ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.

Results determined automatically by the software in total duration of 18 minutes essay by comparing the electrochemiluminescence signal obtained from the reaction product of the sample with the signal of the cutoff value previously obtained by calibration.

B) Test principle HBe Ag

Reagents working solutions

The reagent rack pack (M, R1, R2) is labeled as HBEAG.

M Streptavidin-coated microparticles.

R1 Anti-HBeAg-Ab monoclonal anti-HBeAg antibody

R2 Anti-HBeAg-Ab Monoclonal anti-HBeAg antibody

HBEAG Cal- Negative calibrator

HBEAG Cal- Positive calibrator

Sandwich principle. Total duration of assay: 18 minutes.

▪ 1st incubation: HBe antigen from 35 µL sample, a biotinylated monoclonal HBeAg-specific antibody, and a monoclonal HBeAg-specific antibody labeled with a ruthenium complexa) form a sandwich complex.

▪ 2nd incubation: After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.

▪ The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/ProCell M. Application of a voltage to the electrode then induce chemiluminescent emission which is measured by a photomultiplier.

▪ **Results-** determined automatically by the software by comparing the electrochemiluminescence signal obtained from the reaction product of the sample with the signal of the cutoff value previously obtained by calibration.

Annex IV: Principles of Abbott m2000sp/rt

The Abbott Real Time HBV assay uses PCR to generate amplified product from the DNA genome of HBV in clinical specimens. A DNA sequence that is unrelated to the HBV target sequence is introduced into each specimen at the beginning of sample preparation. This unrelated DNA sequence is simultaneously amplified by PCR and serves as an internal control to demonstrate that the process has proceeded correctly for each sample. The amount of HBV target sequence that is present at each amplification cycle is measured with fluorescent-labeled oligonucleotide specifically bound to the amplified product. The amplification cycle at which fluorescent signal is detected by the Abbott *m*2000*rt* is inversely proportional to the log of the HBV DNA concentration present in the original sample.

Nucleic Acid Extraction: The isolated plasma or serum sample undergoes nucleic acid extraction to isolate the HBV DNA. This step involves breaking open the viral particles and extracting the genetic material.

This process is accomplished by the *m*2000*sp*, an automated sample preparation system designed to use magnetic microparticle processes for the purification of nucleic acids from samples. The assay is suitable for use with both 0.5 mL and 0.2 mL sample input volumes.

reagents lyse the virion, capture the nucleic acids, and wash the particles to remove unbound sample components. Proteinase K is included in the lysis step to digest proteins associated with the nucleic acids. The bound nucleic acids are eluted and transferred to a 96-deepwell plate. The nucleic acids are then ready for amplification.

HBV amplification reagent components (HBV Oligonucleotide Reagent, DNA Polymerase, and Activation Reagent). The Abbott *m*2000*sp* dispenses the resulting master mix to the Abbott 96-Well Optical Reaction Plate along with aliquots of the nucleic acid samples prepared by the Abbott*m*2000*sp*.

Real-Time PCR Amplification: During the amplification/detection reaction the target DNA is amplified by the DNA Polymerase in the presence of deoxynucleotide triphosphates (dNTPs) and magnesium. First, the HBV and IC primers anneal to their respective targets and are extended by the polymerase. After a denaturation step in which the temperature of the reaction is raised above the melting point of the double-stranded DNA product, the newly created DNA strand is denatured from the target DNA. During each round of thermal cycling, amplification products dissociate to single strands at high temperature, allowing primer annealing and extension as the temperature is lowered. Exponential amplification of the target is achieved through repeated cycling between high and lower

temperatures. Amplification of both targets (HBV and IC) takes place simultaneously in the same reaction. The target sequence for the Abbott RealTi*m*e HBV assay is in the Surface gene in the HBV genome. This region is specific for HBV and is highly conserved. The primers are designed to hybridize to this region with the fewest possible mismatches among HBV genotypes A through H. The IC target sequence is derived from the hydroxy pyruvate reductase gene from the pumpkin plant *Cucurbita pepo*, and is provided as a DNA plasmid in a buffer solution.

Detection: The presence of HBV amplification products is detected during the extension/anneal step by measuring the fluorescence of the HBV probe that binds to the target during the extension/anneal step. Similarly, the presence of IC amplification is detected during the extension/anneal step by measuring the fluorescence of the IC probe. The HBV and IC probes are single-stranded DNA oligonucleotides consisting of a probe sequence with a fluorescent moiety that is covalently linked to the 5' end of the probe and a quenching moiety that is covalently linked to the 3' end of the probe. In the absence of the HBV or IC target sequences, probe fluorescence is quenched. In the presence of HBV or IC target, the HBV or IC probes specifically bind to their target. During the extension/anneal step, the DNA polymerase cleaves, or nucleolytically digests, the bound probe as it moves along the template strand. This separates the fluorophore from the quencher, allowing fluorescent emission and detection. The HBV and IC probes are each labeled with a different fluorophore, thus allowing for simultaneous detection of both amplified products at each cycle. The amplification cycle at which fluorescent signal is detected by the Abbott *m*2000*rt* is inversely proportional to the log of the HBV DNA concentration present in the original sample. The Abbott *m*2000*rt* instrument automatically reports the results on the *m*2000*rt* workstation. Assay results are reported in IU/mL or log IU/ml. Results can also be reported in copies/ml or log copies/ml using a conversion factor of 3.41 (1 IU = 3.41 copies).

Annex V: Information Sheet (English Version)

Addis Ababa University College of Health Science, Department of Medical Laboratory Sciences,

Dear Participant,

My name is Kidist Birhanu, A MSC student at Addis Ababa University, College of Health Science, Department of Medical Laboratory Sciences, I am to conduct a study and collect data on Mother to Child Transmission of Hepatitis B Virus and Associated Factors in a government Hospital, Addis Ababa Ethiopia.

The aim of the study is to determine the mother to Child Transmission of Hepatitis B Virus and Associated Factors, your participation is voluntary, and you are free to withdraw your consent and to discontinue participation at any time without consequence. Your participation or not, will not have any influence on the service you receive from the health facility.

Procedure

If you accept to participate for yourself and on behalf of your child, we are going to take about 10 ml of blood from you during 3rd trimester of pregnancy and as early as possible. Moreover, we will also take 5 ml of umbilical blood at birth.

Benefit/risk

The information you provide will be used to improve the quality of prevention MTCT transmission of HBV and also help for national guideline development. The study will identify gaps and challenges and provide recommendations for proper interventions of government and concerned stakeholders for better management of MTCT of HBV.

If you decide to participate, I will guarantee that there is not any influence related to study but only request you that to provide all relevant information regarding the study. I cannot guarantee, however, that you will receive any direct benefits from this study. However, this information will be useful to the management of MTCT of HBV and improve the prevention of HBV transmission from MTC.

Confidentiality

The information that you will give and the results from the specimen will be used for this study only all the data I collect will be stored securely electronically Your name was not written on the questionnaire or kept in any other records.

Thank you for your participation.

Contact address of the PI,

Name Kidist Birhanu (principal investigator)

City: Addis Ababa, Ethiopia

Mobile number +251 910 69 40 11

Email: kidistderessa@gmail.com

Annex VI: Consent form: English version

Study title: Mother to Child Transmission of Hepatitis B Virus and Associated Factors in Government Hospital, Addis Ababa Ethiopia: A Serological and Virological Study.

Purpose of the study: study of Mother to Child Transmission of Hepatitis B Virus and Associated Factor.

Your participation in the study is valid to meet the proposed aim of the study. This informed consent will be read to you, please feel free to ask for further clarification on any issue that you may not understand. Your participation in this study is voluntary; you can withdraw from the study at any time and failure to participate in this study will not affect the services you receive at the hospital. You are being asked to participate in this study because you are HBV positive pregnant mother, attending ANC service at this Hospital.

Procedure

We are providing information to you about this study and would like to invite you to be part of this study. If you accept to participate, we are going to take about 5 ml of blood also take 5 ml of umbilical blood at birth.

Confidentiality

Any information obtained will be kept confidential. Information collected from you will be stored securely and only the researchers will have access to it. Also, we will not use your name on any part of the study; we will use an identification number that will be assigned to you. After data collection we will prepare a report which might be shared at conferences and publications, but this report will not identify you in any way. Your confidentiality in participating in this research study is completely assured.

Certificate of consent

I have read the informed consent, or it has been read to me. I have had the opportunity to ask questions about it and all the questions I have asked have been answered to my satisfaction. I agree to participate in this research on a voluntary basis.

_____ _____ _____

Name of participant Date Signature

If illiterate;

I have witnessed the accurate reading of the consent form to the potential participant and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely

_____ _____ _____

Name of participant Date Thumb print of participant

_____ _____ _____

Name of the witness Date Signature

Statement of the person taking consent

I confirm that the participant was given an opportunity to ask questions about the study, and all the questions asked have been answered correctly and to the best of my ability. I also confirm that the individual has not been coerced into giving consent and the consent has been given freely and voluntarily.

_____ _____ _____

Name of the person taking consent Date Signature

Annex VII-: የተሳትፎ ስምምነት (consent form)

የጥናቱ ርዕስ " Mother to Child Transmission of Hepatitis B Virus and Associated risk Factor in Government Hospital, Addis Ababa Ethiopia: A Serological and virological study".

የጥናቱ ዓላማ፤ ከእናት ወደልጅ የሚተላለፍ የ የጉበት በሽታ አምጪ ህዋስ (hepatitis B virus) መጠን፡ የሚተላለፍበት ምክንያት። በጥናቱ ላይ እንዲሳተፉ የተፈለገበት ምክንያት እርስዎ ከቫይረሱ ጋር የሚኖሩ ነፍሰጡር በመሆኖ እና ወደ ጨቅላው የማስተላለፍ እድል ስላሎት እና በዚህ ሆሰፒታል የቅድመ ወሊድ እና ድህረ ወሊድ ክትትል እየተጠቀሙ ስለሆነ ነው።

በዚህ ጥናት ላይ ለመሳተፍ የስምምነት ፊርማ ነው። ይህን የስምምነት ወረቀት በማንበብ ወይም እንዲነበ ብሎ በማድረግ ያልገባዎት ነገር ካለ ማንኛውንም ጥያቄ የመጠየቅ መብት አለዎት። በዚህ ጥናት ላይ ለመሳተፍ በሙሉ ፈቃድዎ ለይ የተመሰረተ ነው። በፈለጉት ሰዓት ጥናቱን የማቻረጥ መብቶ የተጠበቀ ነው በጥናቱ ላይ አለመሳተፎ ከሆስፒታሉ በሚያገኙት አገልግሎት ላይ ተፅኖ የለውም።

ሂደቶች፤ በጥናቱ ላይ ለመሳተፍ የሚያስፈልጉ መረጃዎችን እየነገርኖ በጥናቱ ላይ እንዲሳተፉ እንጋብዞታለን። ፈቃደኛ ከሆኑ 10ml (ሁለትማንኪያ) የሚሆን ደም እና ህጻኑ እንደተወለደ ከህጻኑ እትብት 5ml (አንድ ማንኪያ) የሚሆን ደም እንወሰዳለን።

ሚስጥራዊነት፡ የሚሰጡት መረጃ ሁሉ ሚስጥራዊ በሆነ ሁኔታ የሚያዙ መሆናቸዉ እንዲሁም አጥኚዎቹ ብቻ የሚያዉቁት ይሆናል። በጥናቱ ላይ ስምዎ የማይገለጽ ሲሆን ለመለያ የሚሆን መለያ ቁጥር የሚሰጦ ይሆናል።

ጥናቱ ከተጠናቀቀ በሃላ ሪፖርት በተለያዩ ኮንፈረንሶች ላይ እንዲሁም በህትመት መልክ የሚሰራጩ ሲሆን ማንነቶን የሚገልጽ ምንም አይነት መረጃ አይኖርም። መረጃው ሚስጥራዊነቱ የተጠበቀ ነው።

ከጥናቱ ጋር ተያያዝ የሆነ ጥያቄ ካሎት

□□□□ □□□□

አድራሻ: አዲስ አበባ ኢትዮጰያ

ስልክ ቁጥር: ሞባይል +251 910694011

የተሳትፎ የምስክር ፊርማ

ይህን የተሳትፎ ስምምነት ፎርም አንብቤ/ተነቦልኝ ተረድቻለሁ። ጥያቄዎችን የመጠየቅ መብት ተሰቶኝ እንዲሁም የጠየኩዋቸው ጥያቄዎች በአጥጋቢ ሁኔታ ተመልሰውልኛል። በጥናቱ ላይ በፍላጎቴ ተስማምቼ ተሳትፊያለሁ።

_____ _____ _____

ስም ፊርማ ቀን

ማንበብ ለማይችሉ ምስክርነት

መጠይቁ በአግባቡ ተነቦላቸው እንዲሁም የመጠየቅ አጋጣሚ ተሰጥቶዋቸዋል። ይህ መፈጸሙን ምስክር መሆኔን በፊርማዬ አረጋግጣለሁ። ግለሰቡ የተሳትፎ ስምምነቱን በነጻነት ነው የፈጸሙት።

_____ _____ _____

የተሳታፊ ስም ቀን የአሻራ ፊርማ

_____ _____ _____

የምስክር ስም ቀን ፊርማ

የጠያቂው ቃል

ለጥናቱ ተሳታፊ ጥያቄ የመጠየቂያ እድል ተፈጥሮላቸዋል በተጨማሪም በምችለው መጠን ጥያቄዎቹ በትክክል ተመልሶላቸዋል። ተጠያቂዉ ጥያቄዎቹን እንዲመልሱ አልተገደዱም መልሱን የሰጡት በራሳቸው ፈቃድ እና ነጻነትነው።

_____ _____ _____

የጠያቂው ስም ቀን ፊርማ

Annex VIII: Declaration

The undersigned declares that this thesis complies with the regulations of the University and meets the accepted standards with respect to originality and quality. PI also agrees to accept responsibility for the scientific ethical and technical conduct of the research project and for the provision of required progress reports.

M.Sc. candidate: Kidist Birhanu (BSc.)

Signature: _____

Date of submission: _____

This thesis has been submitted with our approval as advisors.

Advisor: Dr. Abay Sisay (BSc, MSc, PhD)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.

Advisor: Regasa Diriba (BSc, MSc)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.